

U.S.S.N 09/910,120

Ault-Riche *et al.*

PRELIMINARY AMENDMENT ATTACHMENT

MARKED-UP COPY OF FIGURES

Sorting by pools

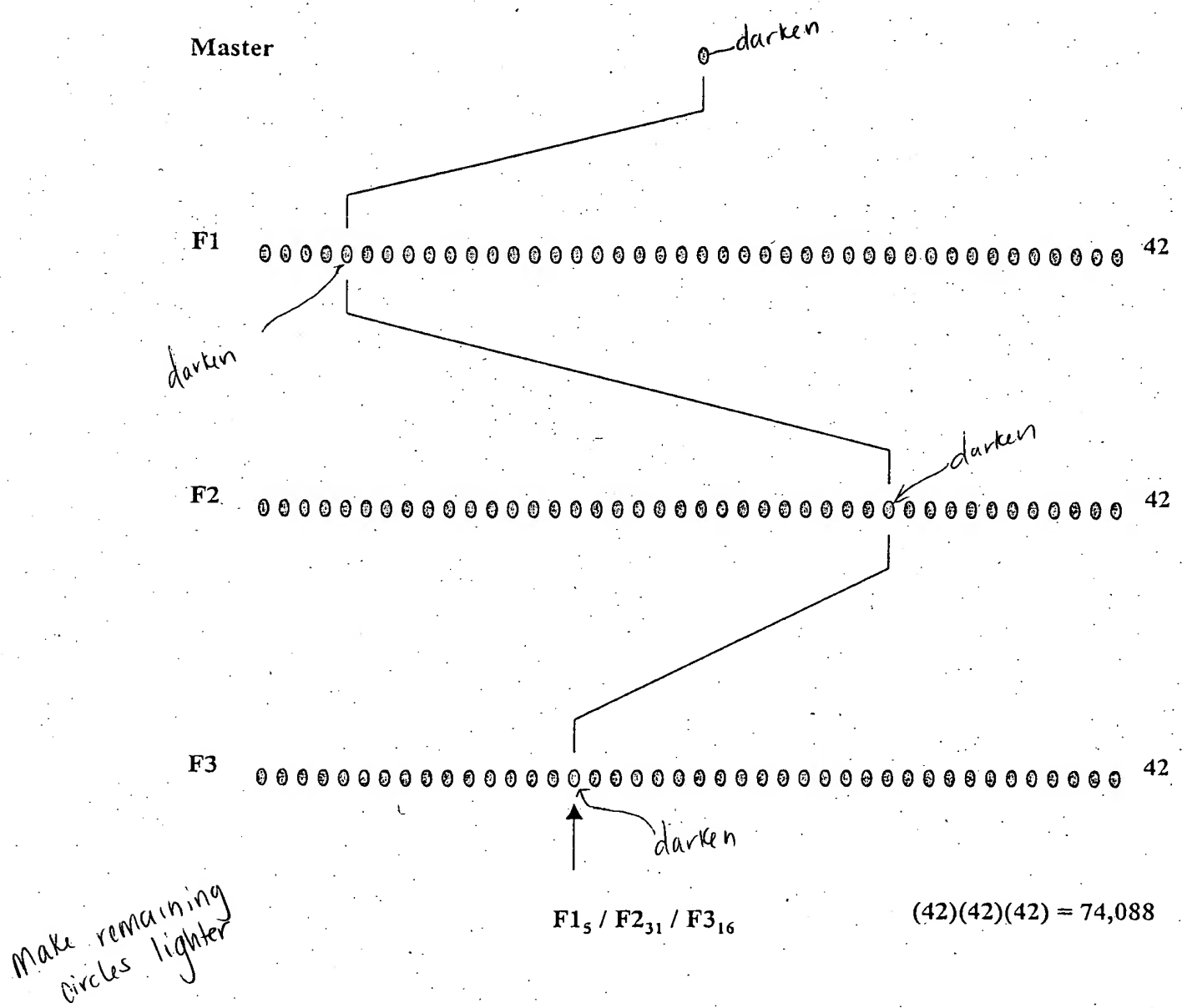


FIGURE 1

Sorting by pools: Decreasing pool diversities

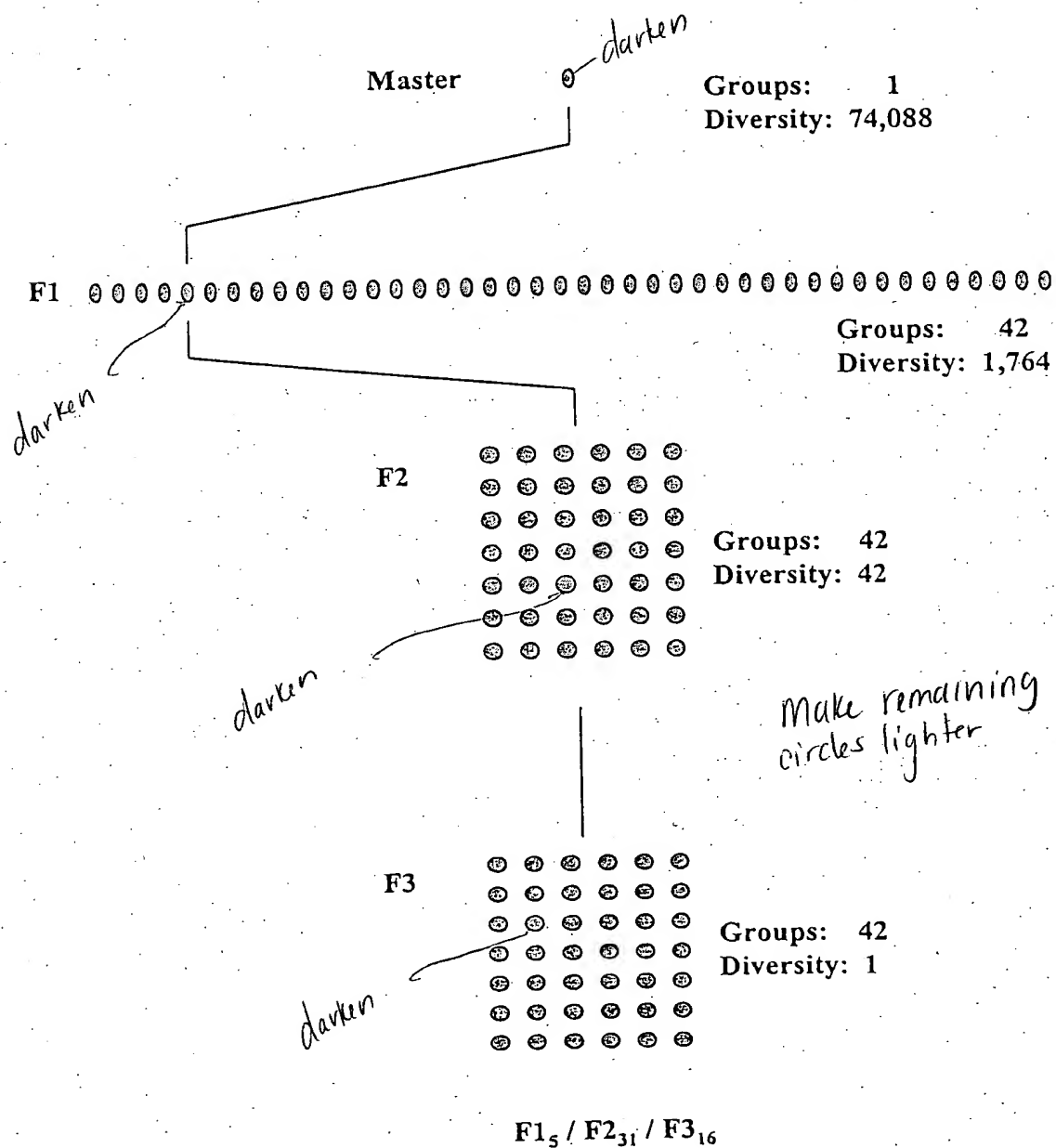


FIGURE 2

Sorting by pools: Screening large diversity libraries

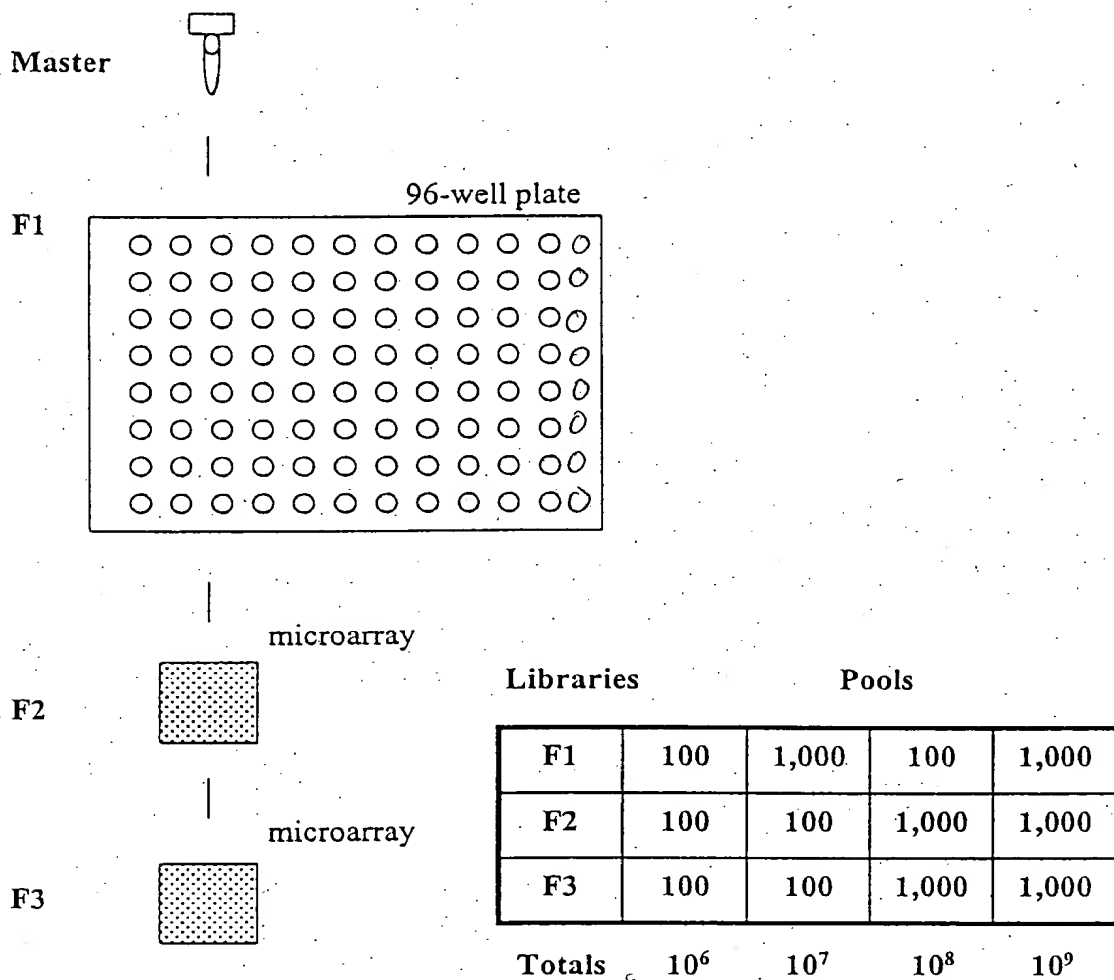


FIGURE 3

Searching a mutation library

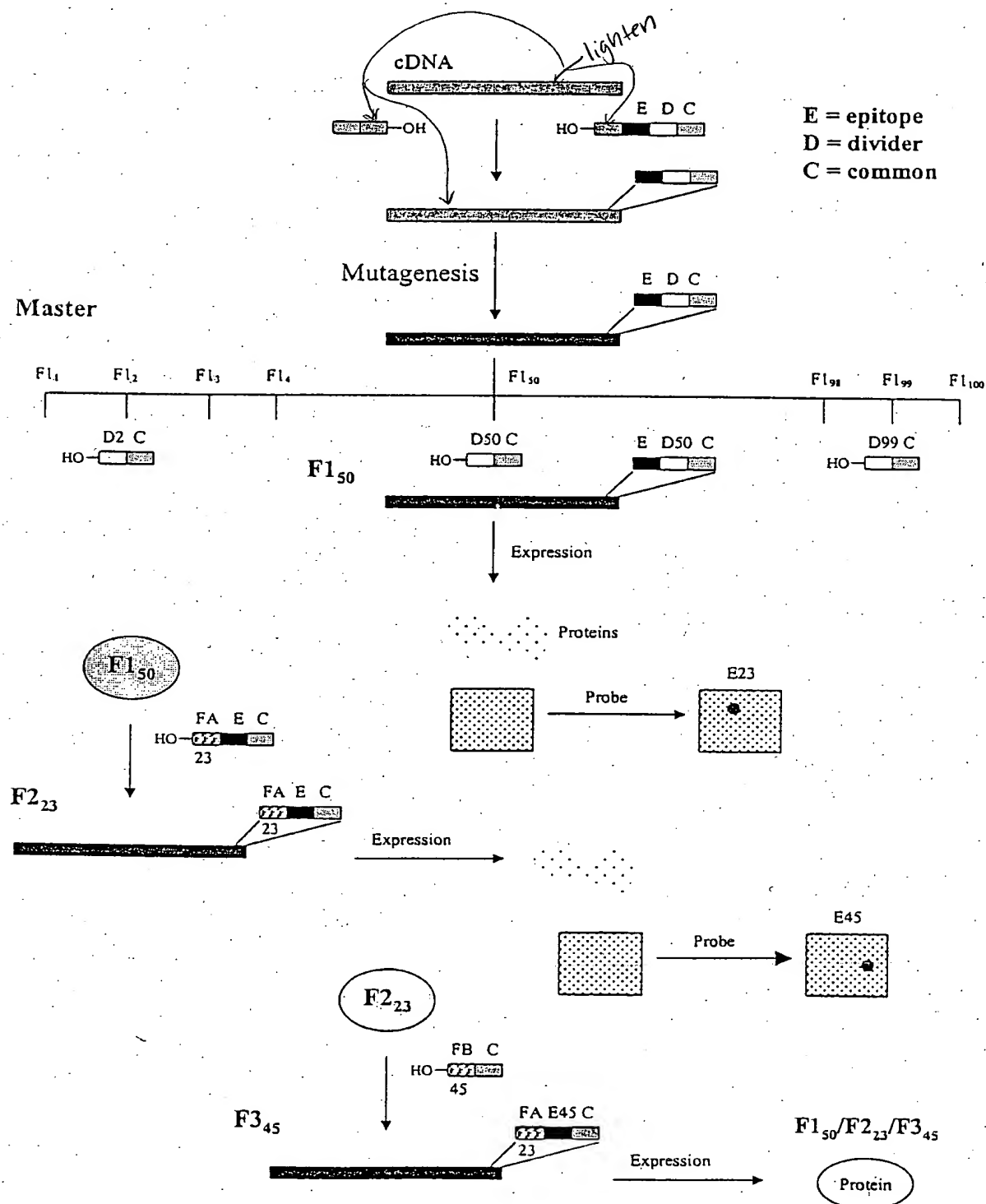
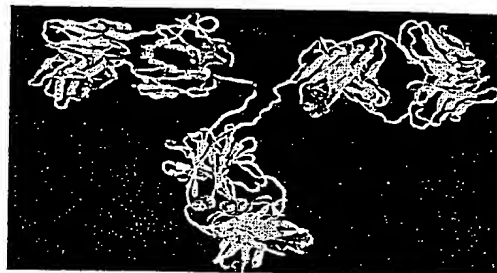
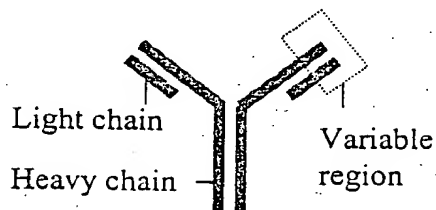


FIGURE 4

Applicant: Ault-Riche *et al.*
DKT. No. 25885-1751
Priority claimed to 60/219,183
For: COLLECTIONS OF BINDING PROTEINS AND
TAGS AND USES THEREOF FOR NESTED SORTING
AND HIGH THROUGHPUT SCREENING

Making a recombinant antibody library

Basic antibody structure



Spleen cells or PBLs

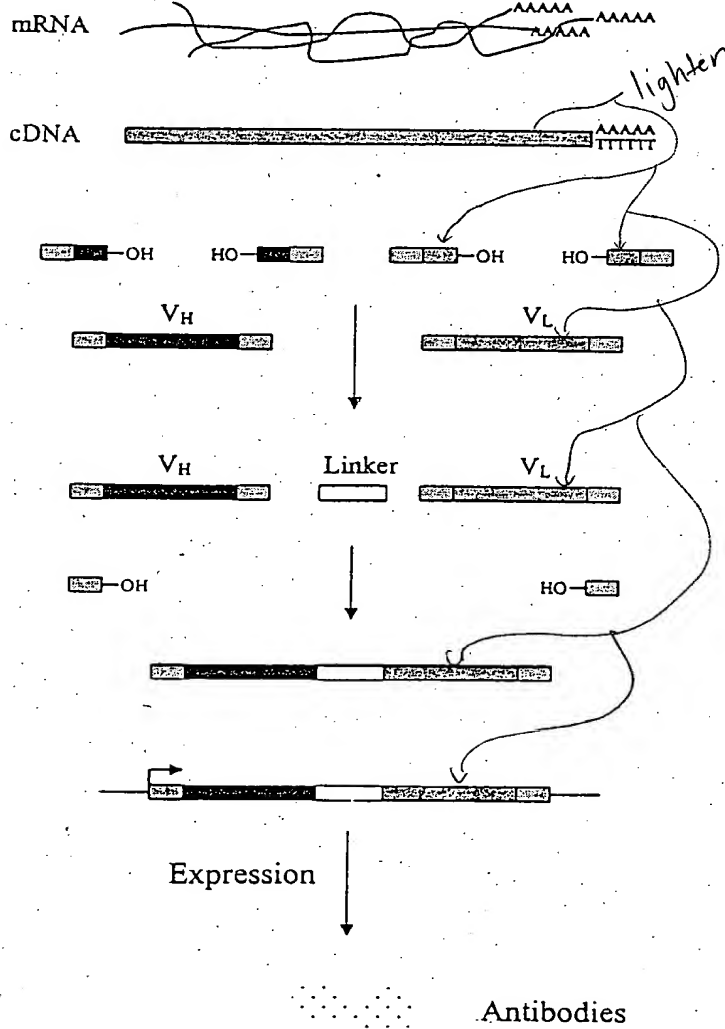
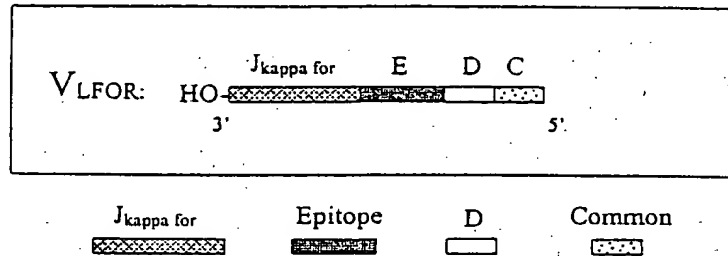


FIGURE 5

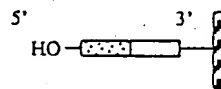
The diagram illustrates the iterative construction and screening of a phage display library. It begins with a 'Master' phage DNA containing various antibody gene fragments (V_H, V_L, E, D, C) and a 'lighten' label. This is followed by the 'F1' generation, where specific fragments are selected (e.g., D2 C, D50 C, E D50 C, D99 C). The 'F1' phage is then 'Expressed' into 'Proteins'. A 'Probe' is used to select specific proteins (e.g., E23, E45). The selected proteins are then 'Expressed' into 'F2' and 'F3' generations (e.g., F2₂₃, F3₄₅). The final 'F1₅₀/F2₂₃/F3₄₅' library is used to produce an 'Antibody'.

FIGURE 8

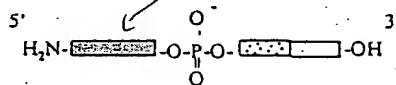
Making the V_LFOR primers: Solid phase synthesis



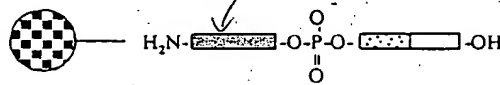
1. Synthesize oligo on solid support



2. Add aminolink prior to cleavage



3. Couple to tosyl activated magnetic beads



4. Extend by hybridizing with DNA patch and ligating

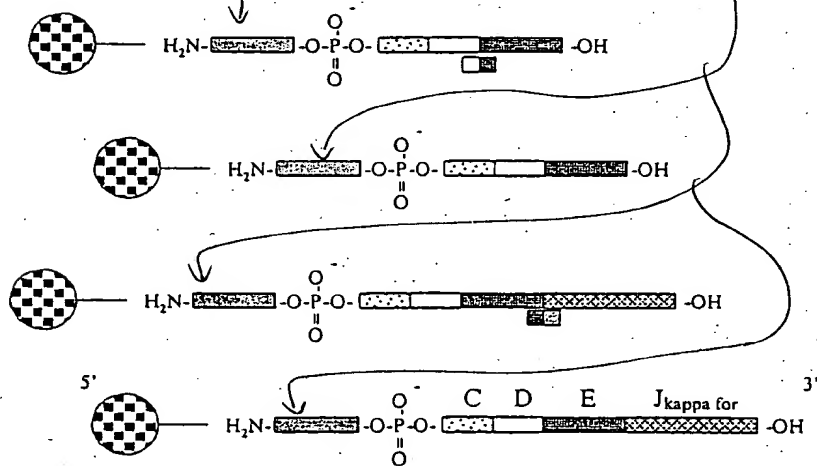
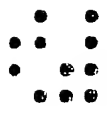


FIGURE 10



step II

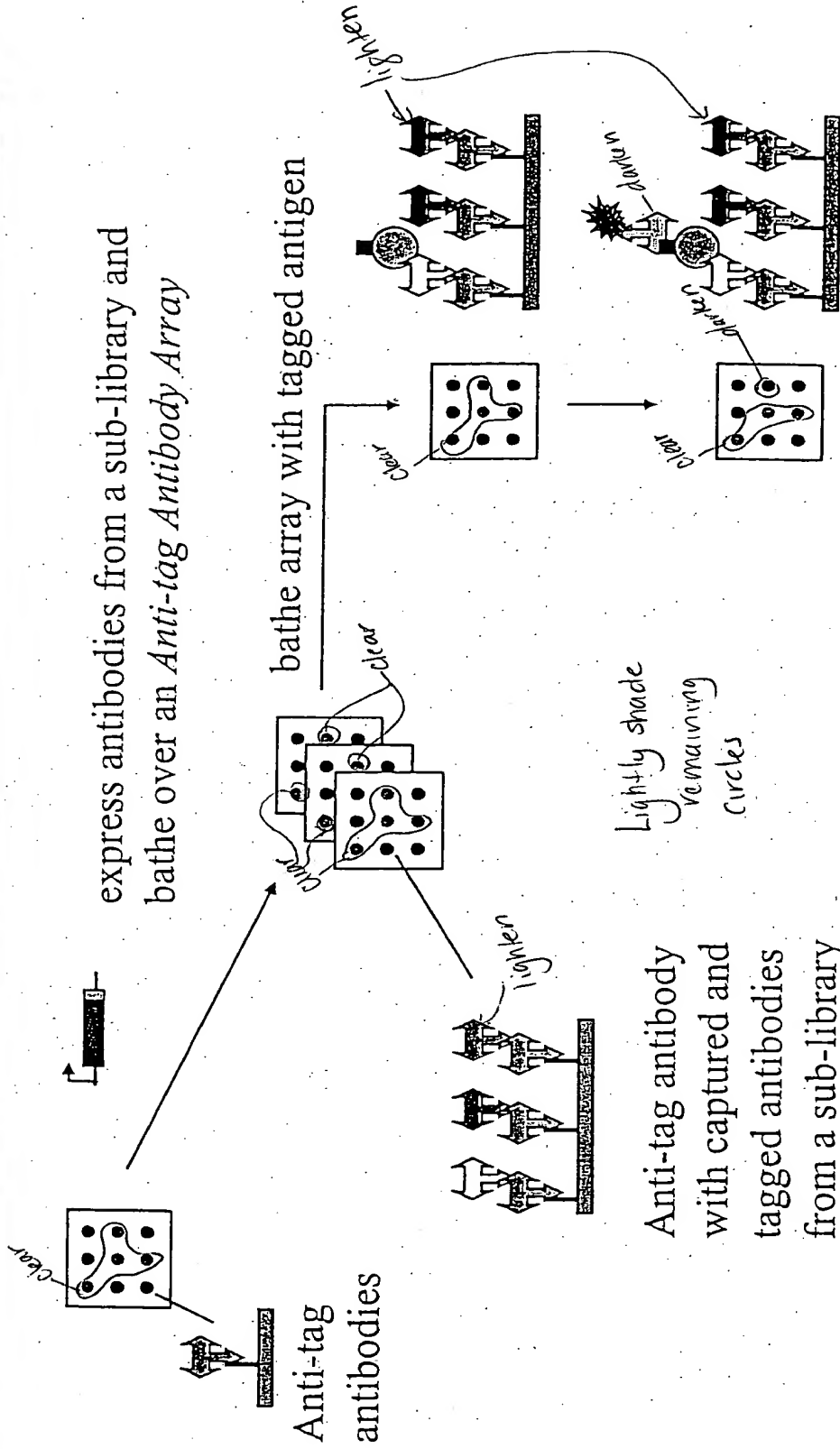
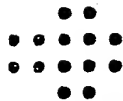


FIGURE 14B



step III

Amplify the antibody genes from the identified sub-library using tag-specific PCR primers

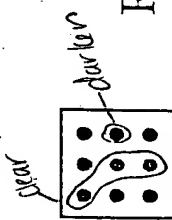
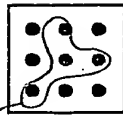
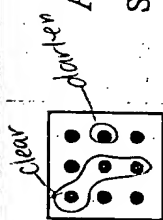
If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have 1,000 different types of rAbs

Express and purify the antibodies

Re-distribute over an *Anti-tag Antibody Array*

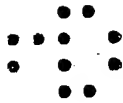
If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have a single type of rAb

Re-survey to ID the antibody of interest



Lightly shade remaining circles

FIGURE 14C

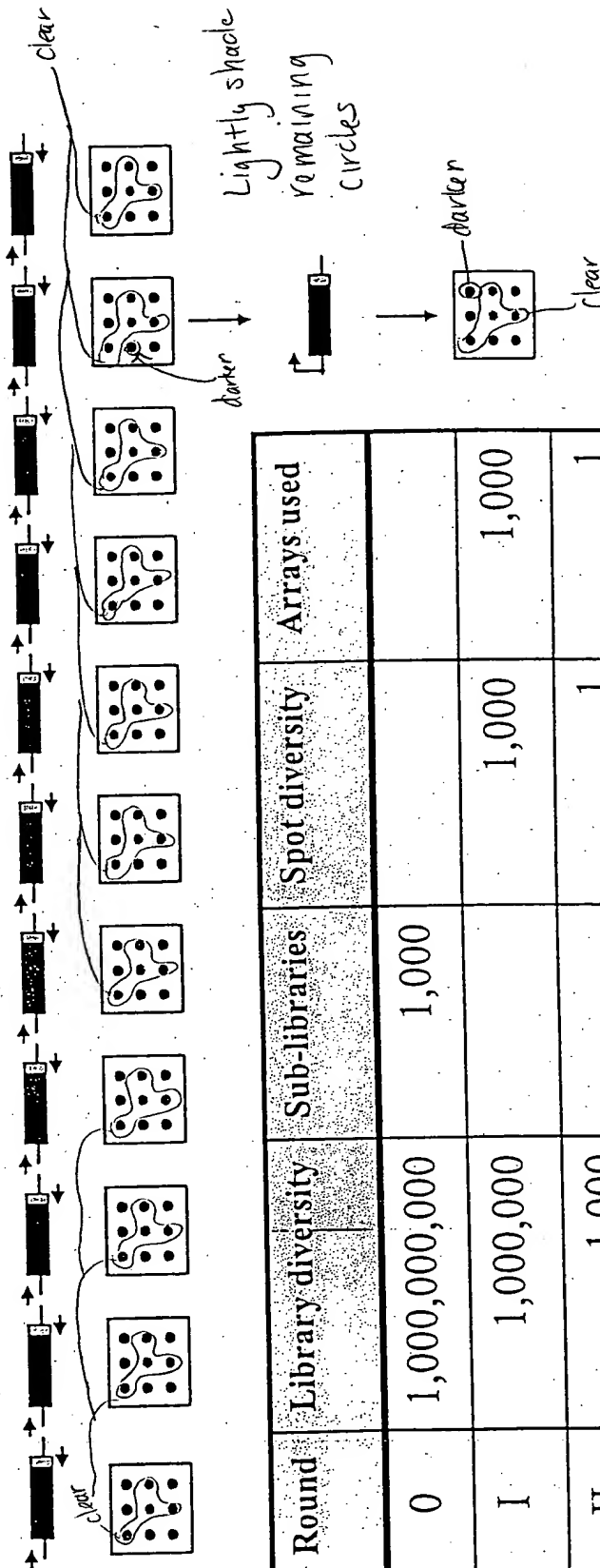


summary

master library

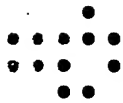
1,000 sub-libraries

HELLER EHRMAN WHITE & McALIFFE LLP
 Sheet 18 of 23
 Applicant: Auli-Riche et al.
 DKT. No. 25885-1751
 Priority claimed to 60/219,183
 For COLLECTIONS OF BINDING PROTEINS AND
 TAGS AND USES THEREOF FOR NESTED SORTING
 AND HIGH THROUGHPUT SCREENING



Round	Library diversity	Sub-libraries	Spot diversity	Arrays used
0	1,000,000,000	1,000		
I	1,000,000		1,000	1,000
II	1,000		1	1

FIGURE 14D



- Modification searches

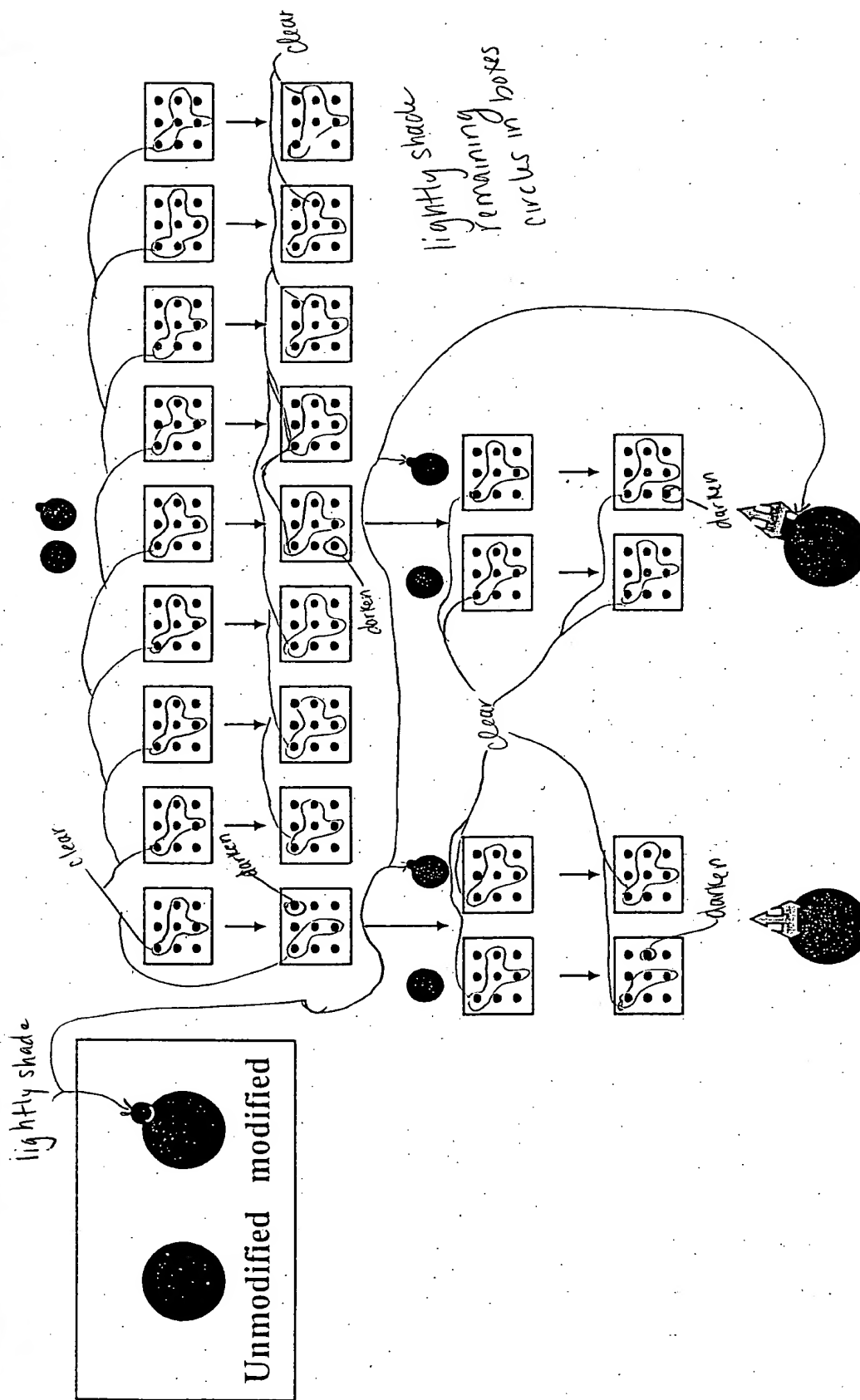


FIGURE 15

Simultaneous searches

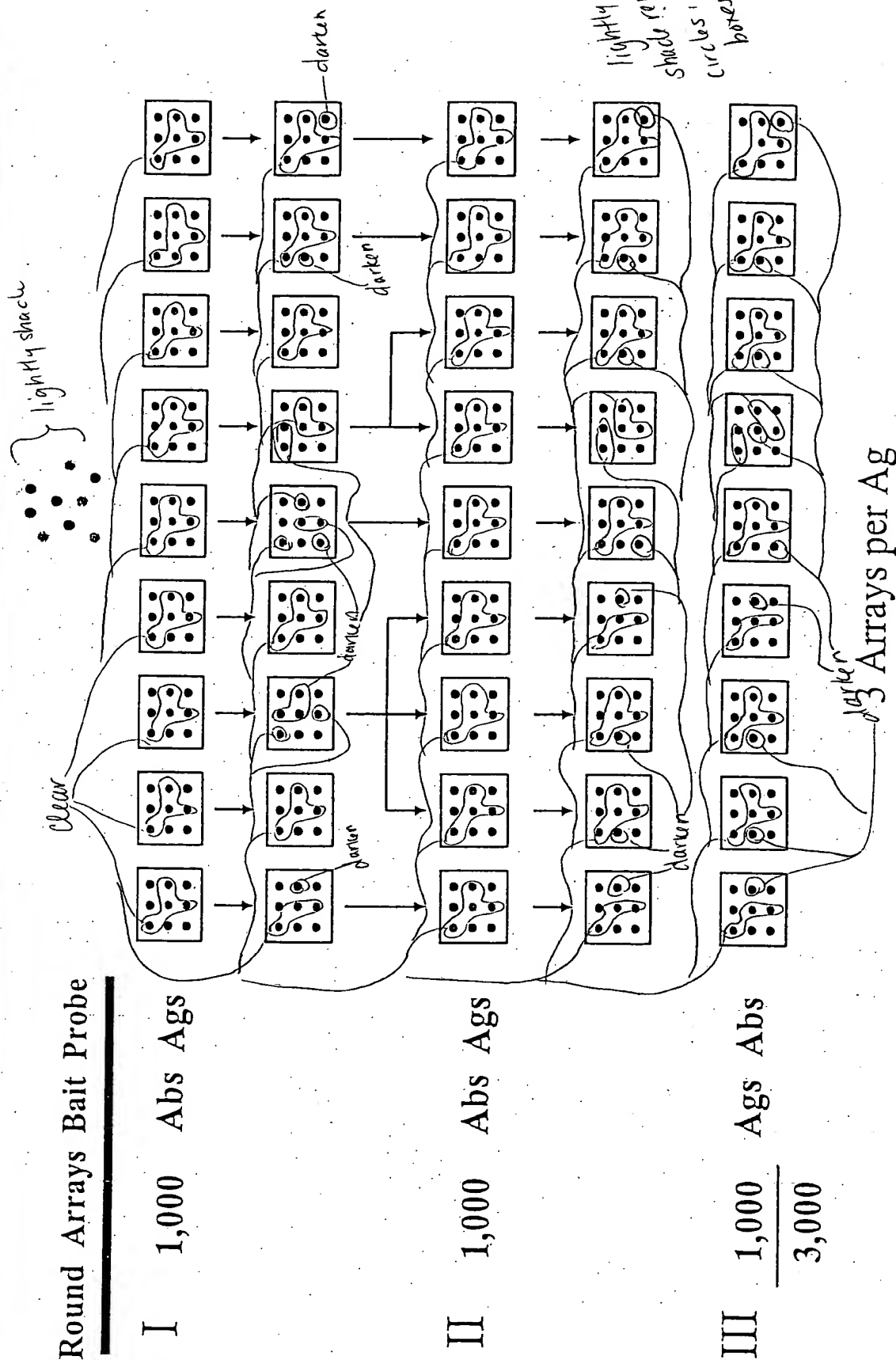


FIGURE 16

Enzyme engineering

Error-prone PCR
or Gene Shuffling

Natural gene(s)

Mutated genes



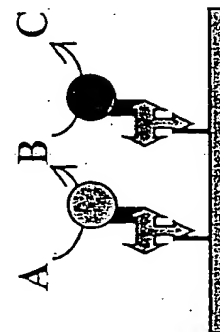
- tag the gene to be mutated

- mutate genes and create sub-libraries

- distribute mutants over arrays

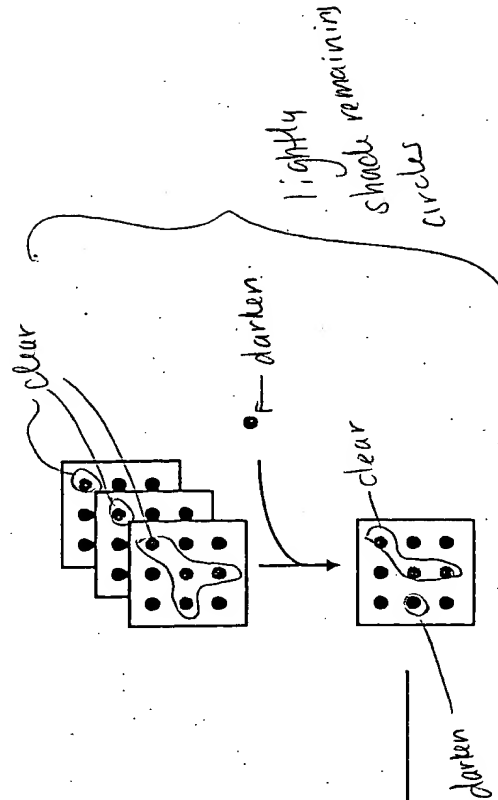
- probe the arrays with labeled substrates

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Sheet 21 of 23
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AND HIGH THROUGHPUT SCREENING



Spots can contain mixtures of enzymes
for detection or pathway engineering

FIGURE 17



Protein interaction mapping

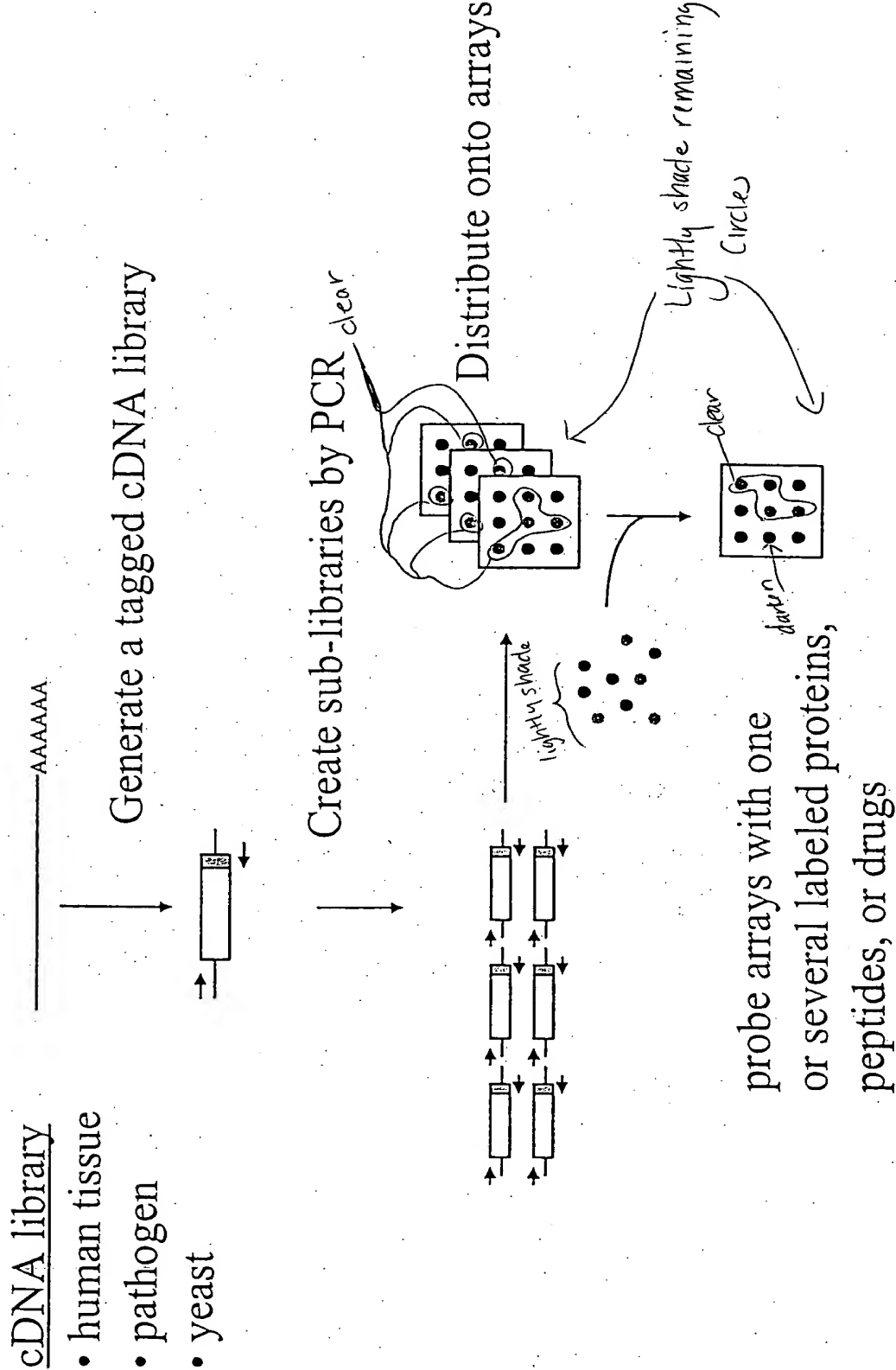


FIGURE 18





FIG. 2

Sorting by pools: Screening large diversity libraries

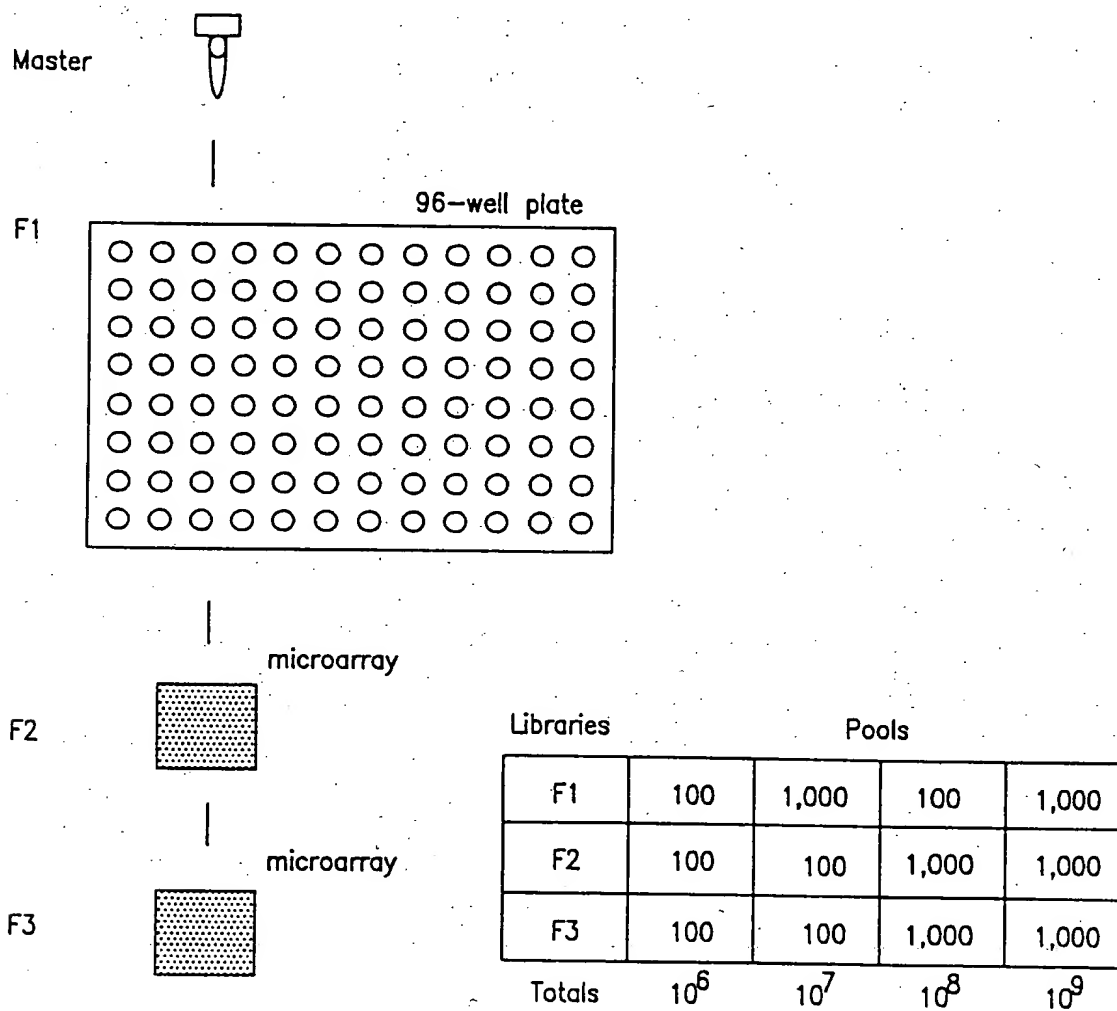


FIG. 3

Searching a mutation library

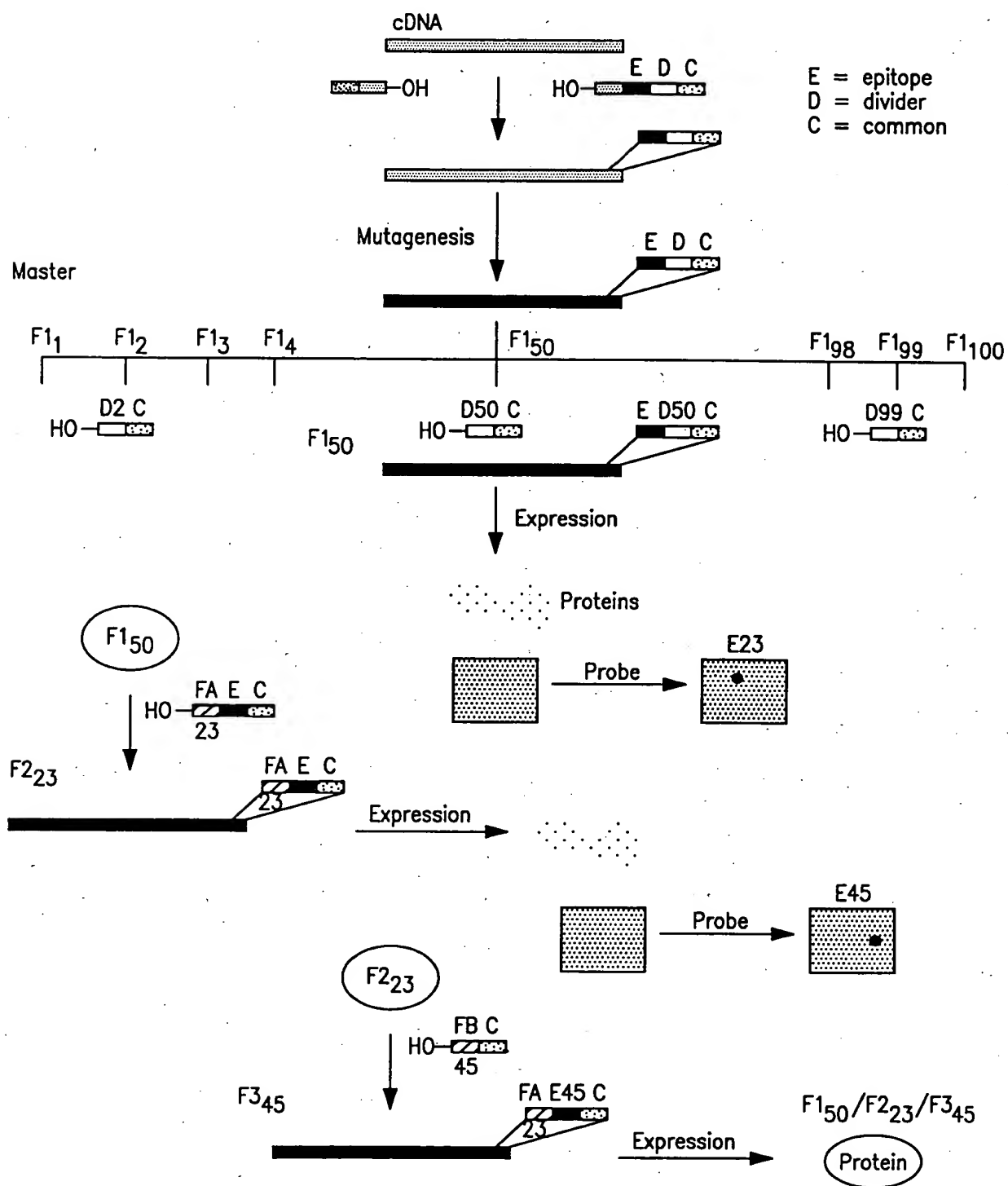
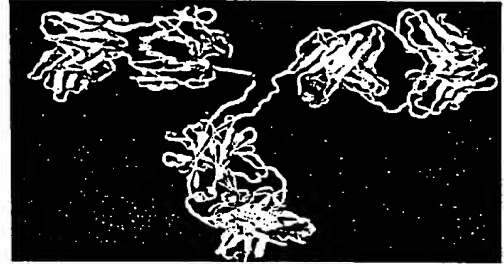
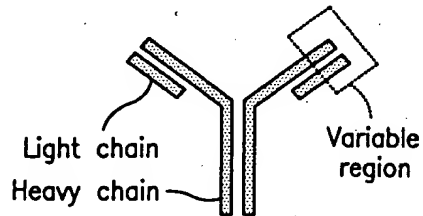
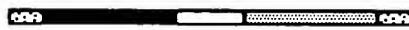
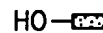
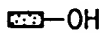
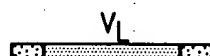


FIG. 4

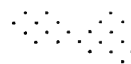
Making a recombinant antibody library



Spleen cells or PBLs



Expression



Antibodies

FIG. 5

Searching a recombinant antibody library

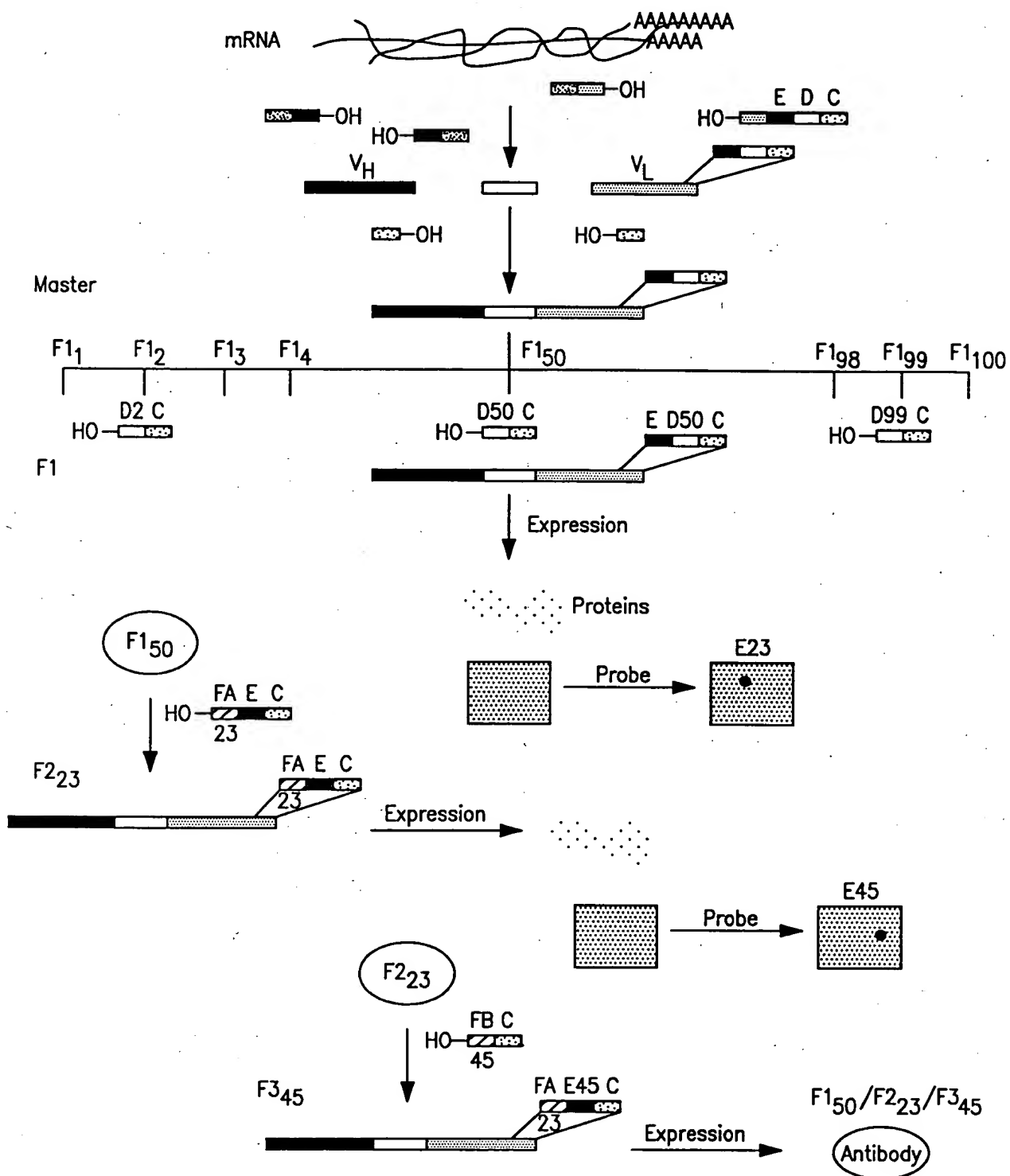
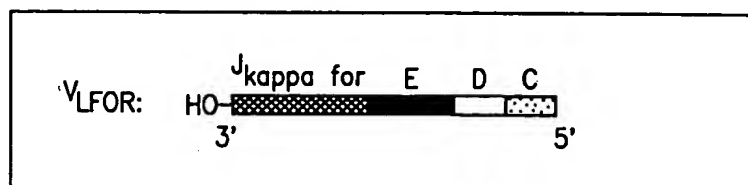


FIG. 8

Making the V_LFOR primers: Solid phase synthesis



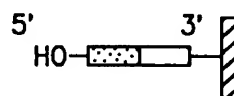
Jkappa for


Epitope

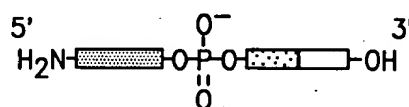

D


Common

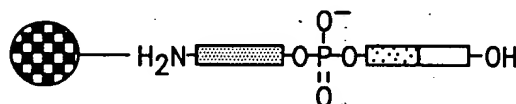

1. Synthesize oligo on solid support



2. Add aminolink prior to cleavage



3. Couple to tosyl activated magnetic beads



4. Extended by hybridizing with DNA patch and ligating

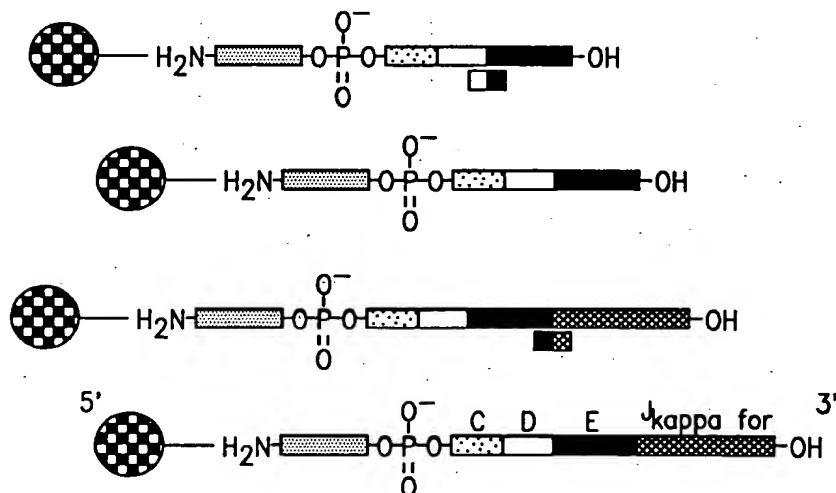
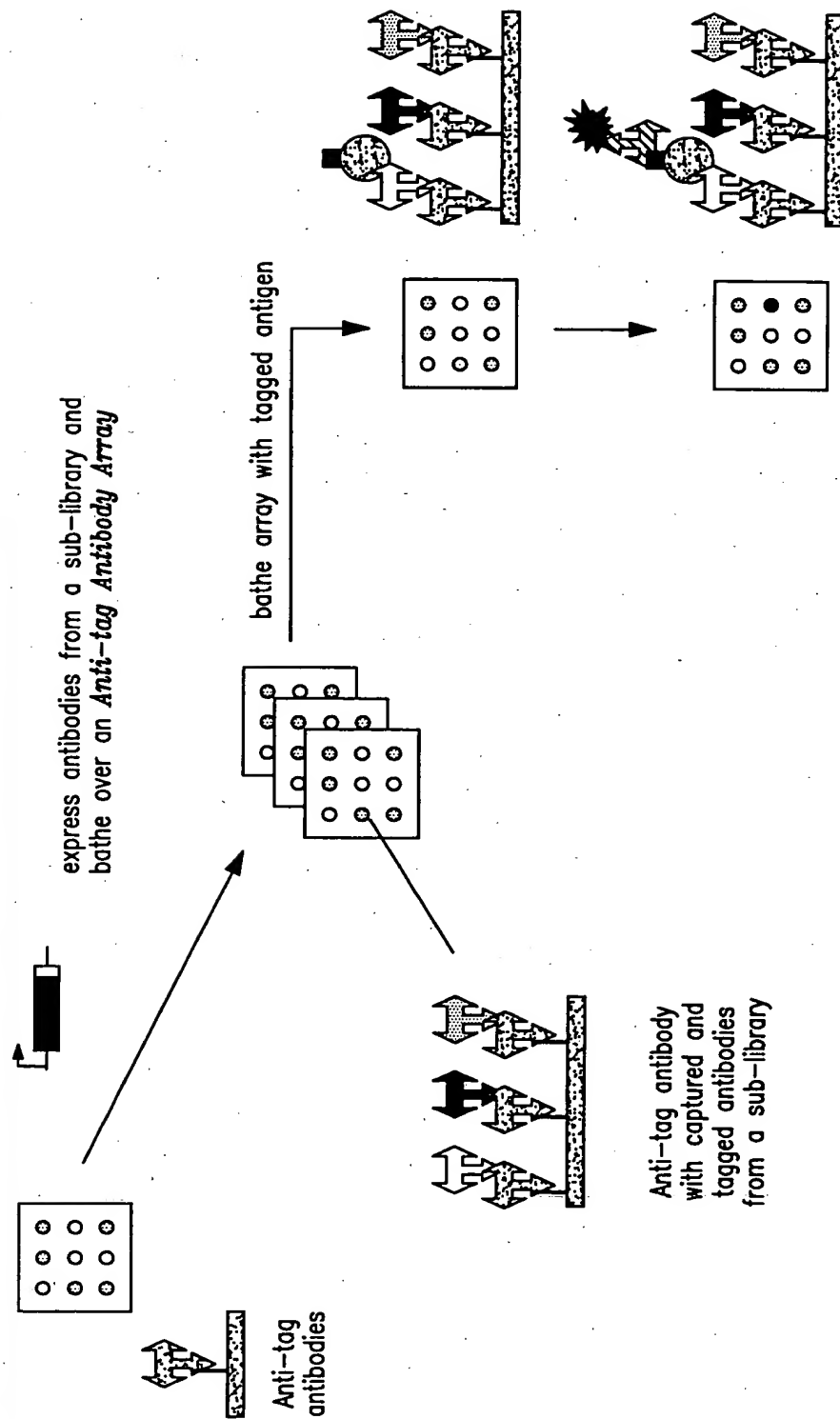


FIG. 10

step II



ID spot containing the antigen
with a labeled developing Ab

FIG. 14B

step III

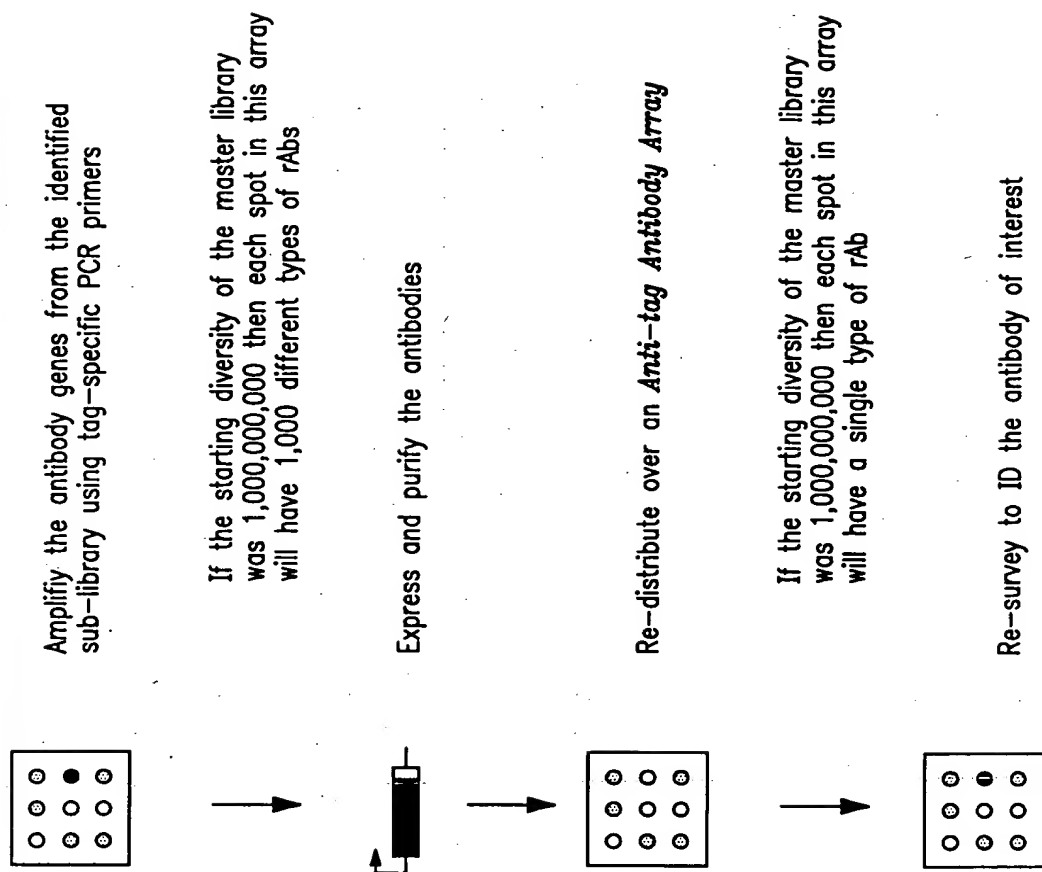
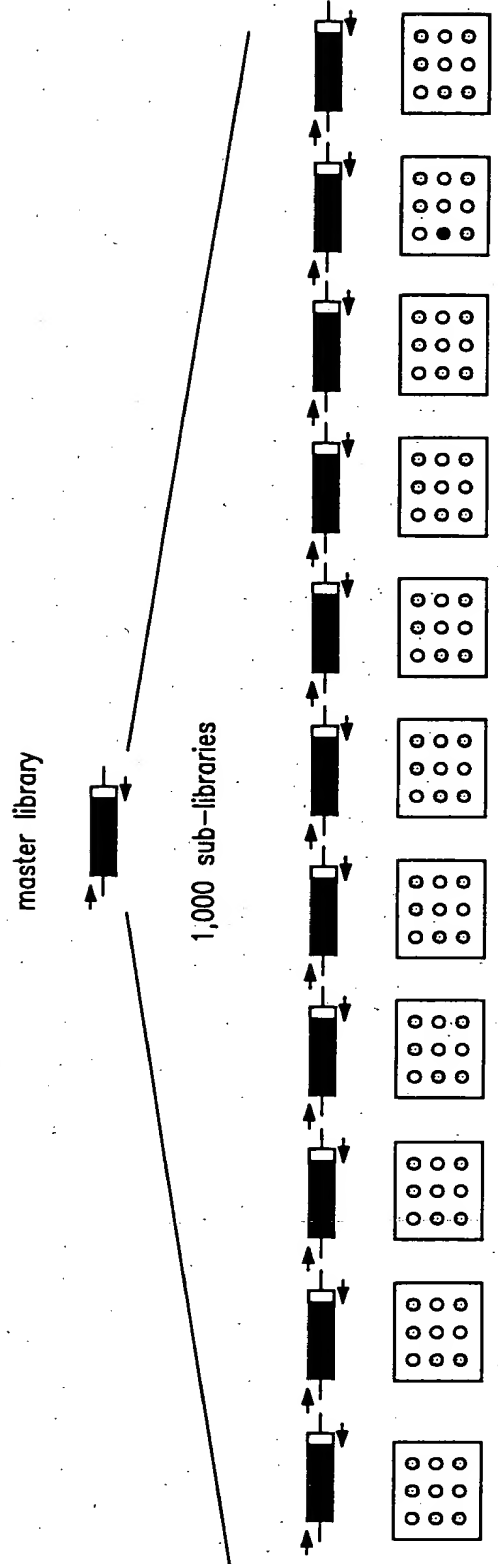


FIG. 14C

...

summary



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FIG. 14D

Modification searches

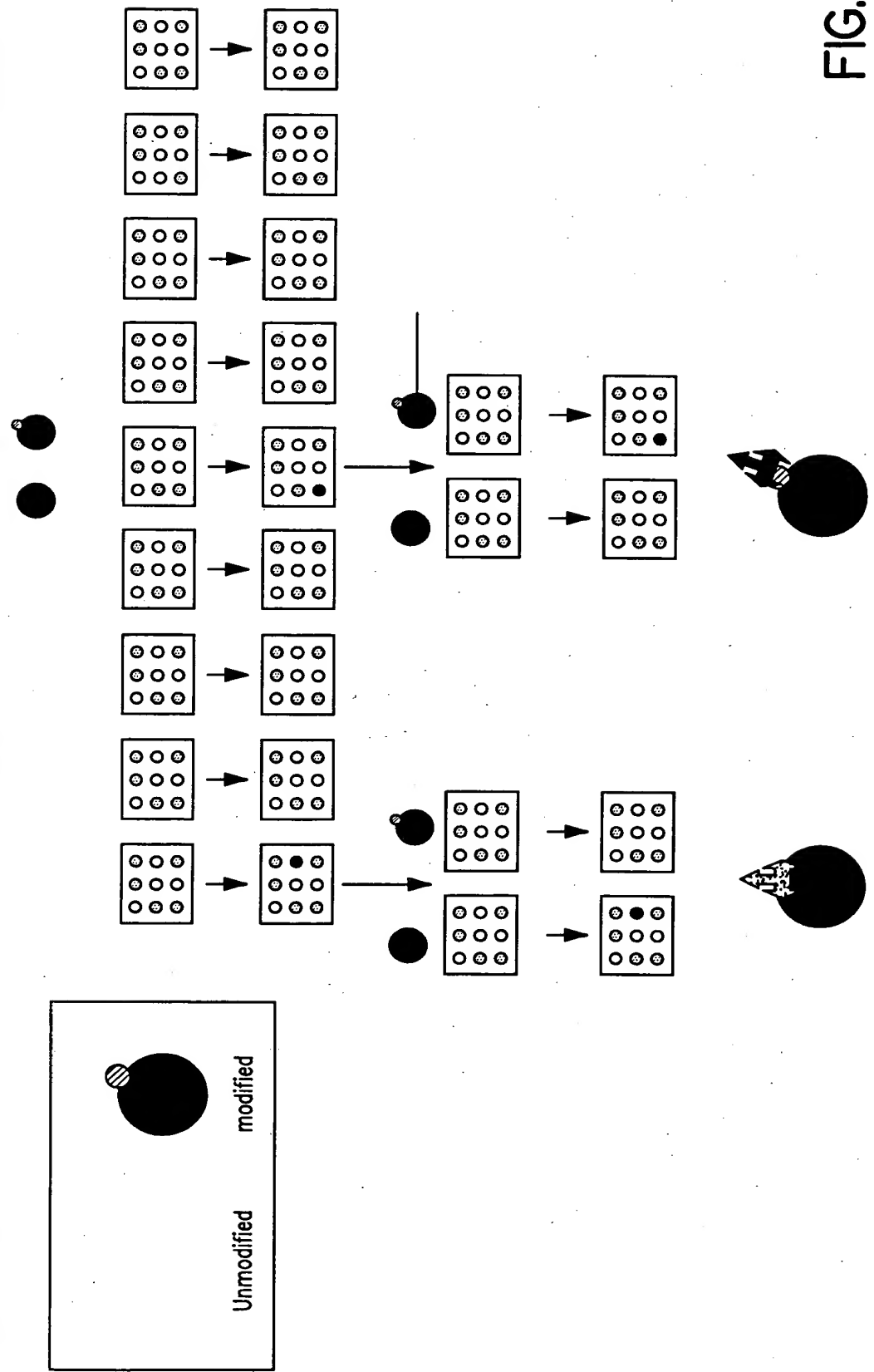
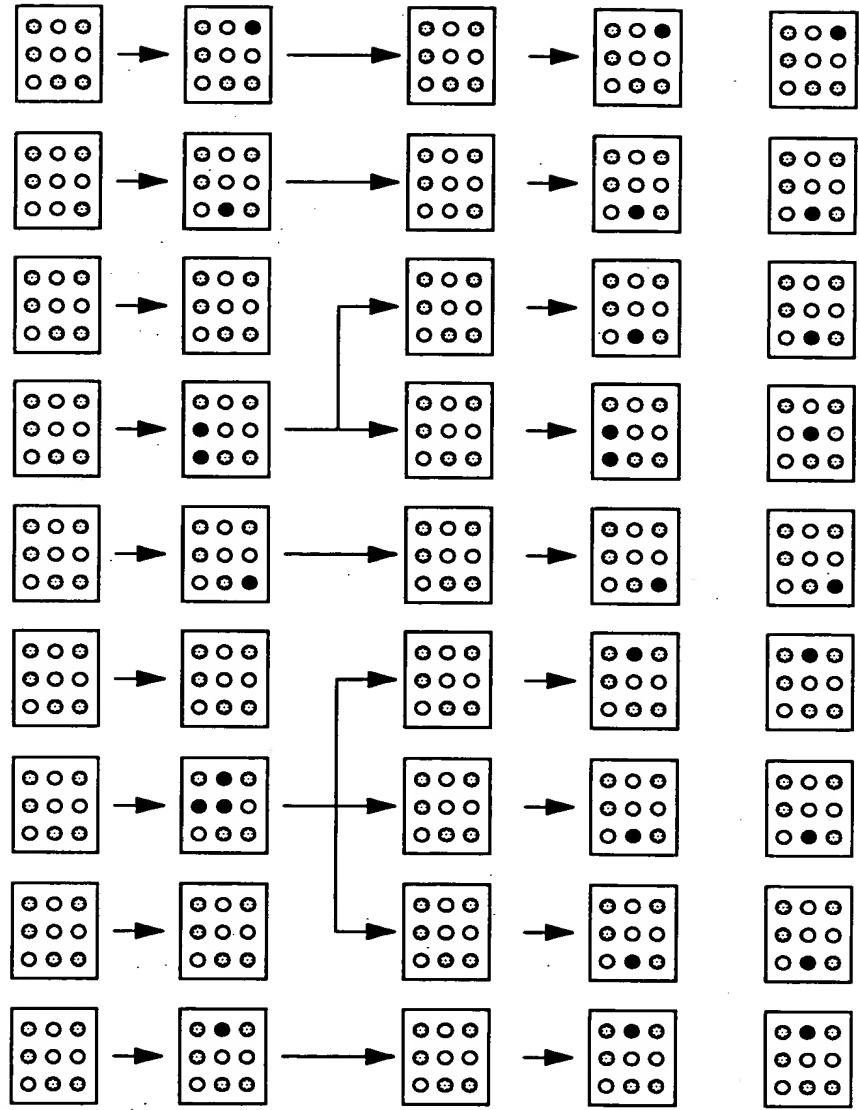


FIG. 15

Simultaneous searches

Round Arrays Bait Probe

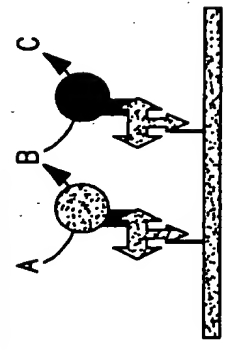
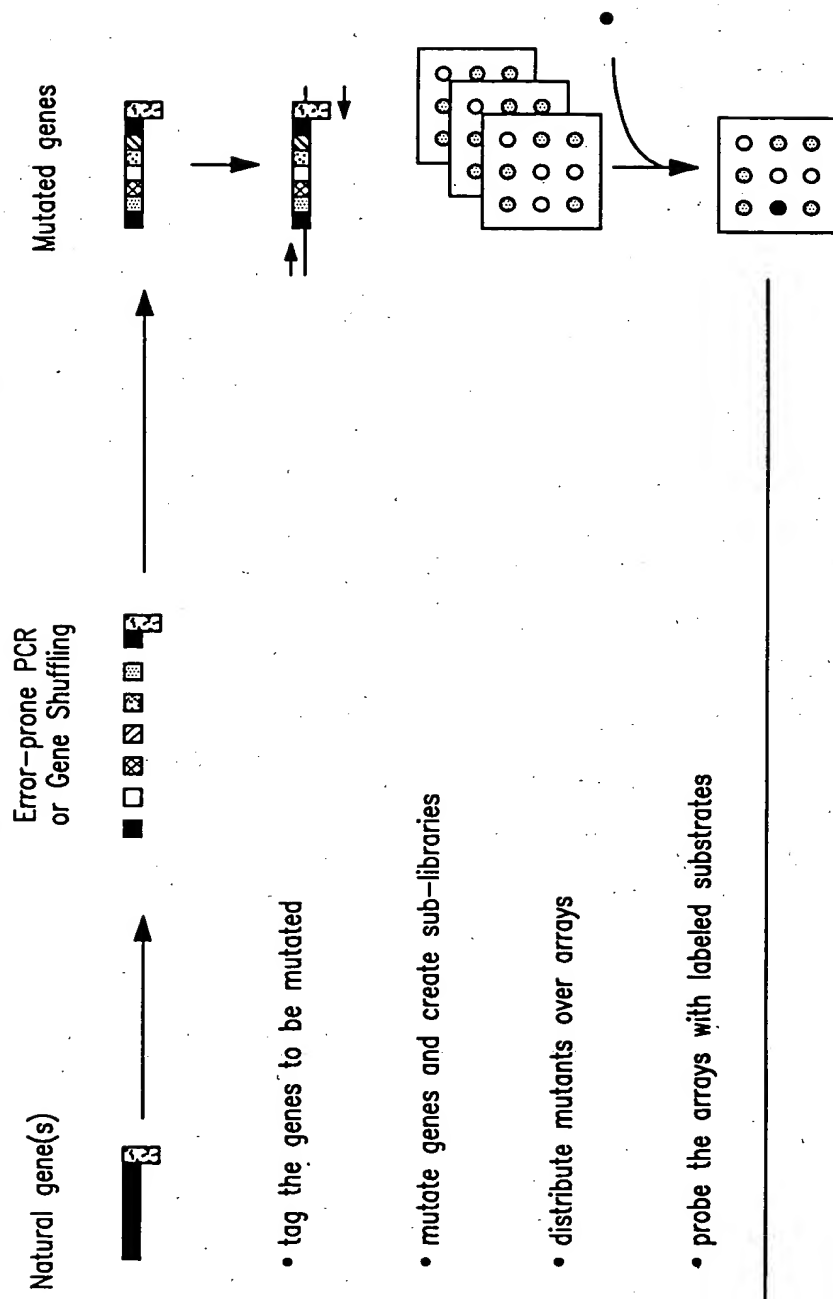


3 Arrays per Ag

FIG. 16

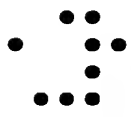
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Protein interaction mapping



Spots can contain mixtures of enzymes for detection or pathway engineering

FIG. 17



Protein interaction mapping

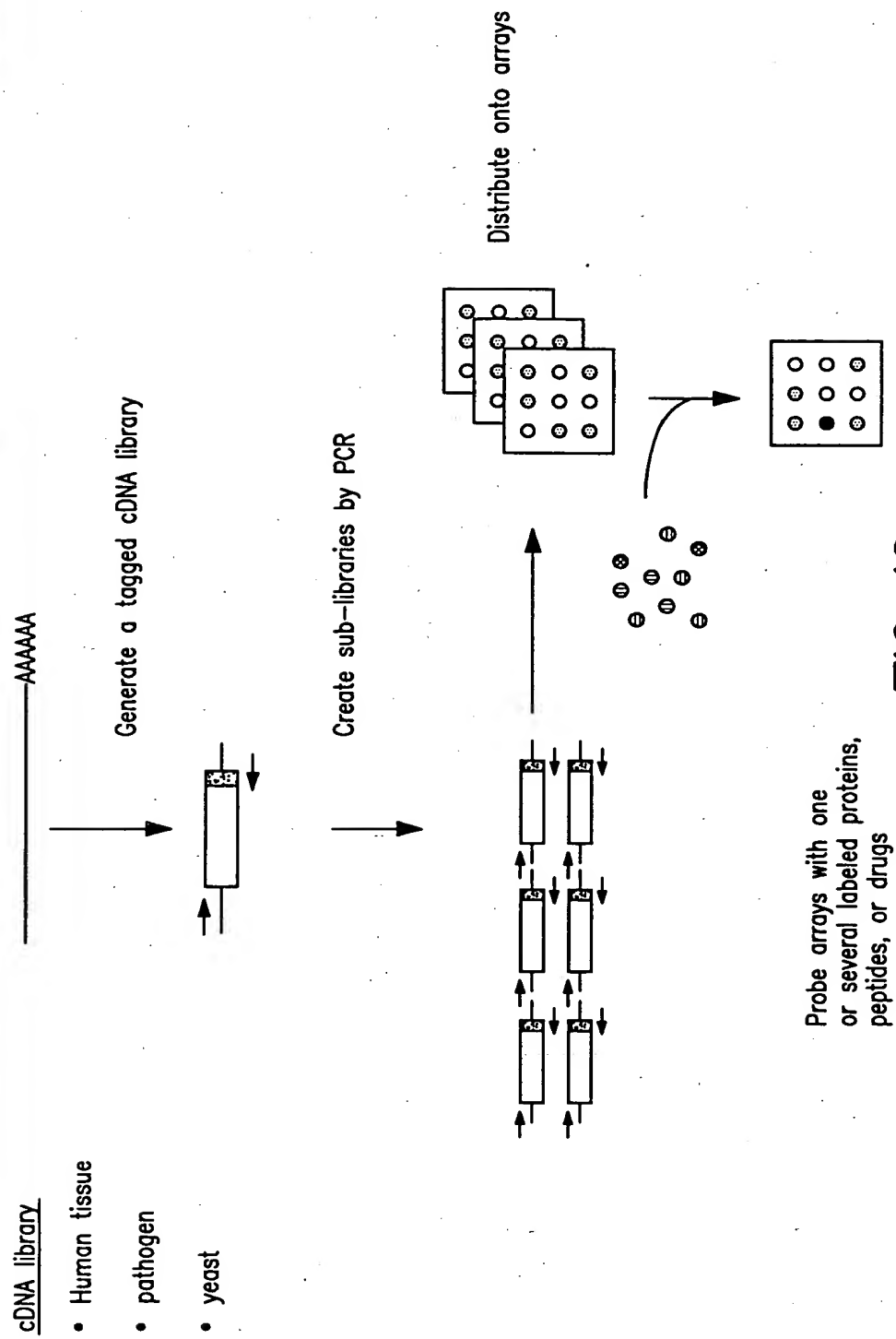


FIG. 18